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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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10/673,409

09/30/2003

Richard Thompson

3645-0114P

2702

2292

7590

04/05/2007

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EXAMINER

SODERQUIST, ARLEN

ART UNIT

PAPER NUMBER

1743

SHORTENED STATUTORY PERIOD OF RESPONSE	NOTIFICATION DATE	DELIVERY MODE
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3 MONTHS

04/05/2007

ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

If NO period for reply is specified above, the maximum statutory period will apply and will expire 6 MONTHS from the mailing date of this communication.

Notice of this Office communication was sent electronically on the above-indicated "Notification Date" and has a shortened statutory period for reply of 3 MONTHS from 04/05/2007.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

mailroom@bskb.com

Office Action Summary

Application No.

10/673,409

Applicant(s)

THOMPSON ET AL.

Examiner

Arlen Soderquist

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-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☐ Responsive to communication(s) filed on ____.
- 2a) ☐ This action is **FINAL**. 2b) ☒ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) 1-20 is/are pending in the application.
- 4a) Of the above claim(s) ____ is/are withdrawn from consideration.
- 5) ☐ Claim(s) ____ is/are allowed.
- 6) ☒ Claim(s) 1-20 is/are rejected.
- 7) ☐ Claim(s) ____ is/are objected to.
- 8) ☐ Claim(s) ____ are subject to restriction and/or election requirement.

Application Papers

- 9) ☒ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on ____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
- Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
- Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All b) ☐ Some * c) ☐ None of:
1. ☐ Certified copies of the priority documents have been received.
 2. ☐ Certified copies of the priority documents have been received in Application No. ____.
 3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- 1) ☒ Notice of References Cited (PTO-892)
- 2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
- 3) ☐ Information Disclosure Statement(s) (PTO/SB/08)
Paper No(s)/Mail Date ____.
- 4) ☐ Interview Summary (PTO-413)
Paper No(s)/Mail Date. ____.
- 5) ☐ Notice of Informal Patent Application
- 6) ☐ Other: ____.

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1. The use of the trademark Alexa Fluor 594 has been noted in this application. It should be capitalized wherever it appears and be accompanied by the generic terminology.

Although the use of trademarks is permissible in patent applications, the proprietary nature of the marks should be respected and every effort made to prevent their use in any manner which might adversely affect their validity as trademarks.

2. The incorporation of essential material in the specification by reference to an unpublished U.S. application, foreign application or patent, or to a publication is improper. Applicant is required to amend the disclosure to include the material incorporated by reference, if the material is relied upon to overcome any objection, rejection, or other requirement imposed by the Office. The amendment must be accompanied by a statement executed by the applicant, or a practitioner representing the applicant, stating that the material being inserted is the material previously incorporated by reference and that the amendment contains no new matter. 37 CFR 1.57(f).

3. The attempt to incorporate subject matter into this application by reference to the cited papers beginning at page 22 is ineffective because as noted above the incorporation by reference of a publication is not proper.

4. Claim 18 is objected to under 37 CFR 1.75(c), as being of improper dependent form for failing to further limit the subject matter of a previous claim. Applicant is required to cancel the claim(s), or amend the claim(s) to place the claim(s) in proper dependent form, or rewrite the claim(s) in independent form. Claim 18 is dependent from claim 17 and contains the identical limitation. It appears the applicant may have intended claim 18 to be dependent from claim 16.

5. Applicant is advised that should claim 17 be found allowable, claim 18 will be objected to under 37 CFR 1.75 as being a substantial duplicate thereof. When two claims in an application are duplicates or else are so close in content that they both cover the same thing, despite a slight difference in wording, it is proper after allowing one claim to object to the other as being a substantial duplicate of the allowed claim. See MPEP § 706.03(k).

6. Claims 5-7 and 11-13 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Alexa Fluor 594 is a trade name or Trademark which identifies the producer of the fluorophore rather than generic terminology that defines the fluorophore.

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7. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

The factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

8. Claims 1-3, 8-9, 14-18 and 20 are rejected under 35 U.S.C. 103(a) as being unpatentable over Thompson (US 5,952,236) in view of Tsien (US 5,439,797). In the patent Thompson teaches a method of detecting zinc ions by disposing carbonic anhydrase modified to have a fluorophore attached thereto in combination with a colored sulfonamide dye in a sample. The fluorescence from the fluorophore being measurable in the absence of zinc. In the presence of zinc, the zinc binds to the carbonic anhydrase and the colored sulfonamide dye binds to the zinc. This brings the dye and indicator into proximity such that fluorescent energy transfer can occur reducing the amount of fluorescence from the fluorophore. A variety of fluorophores are taught as usable for the fluorophore (see column 7, lines 34-50 including fluorescein, rhodamine, Green Fluorescent Protein, CY-3 and CY-5). The paragraph bridging columns 7-8 teaches that many methods can be used including fluorescence lifetime. Figure 1 shows the binding of a fluorescent sulfonamide to the zinc in carbonic anhydrase in a zinc sensing method. Thompson does not teach the colored dye being a donor fluorophore.

In the patent Tsien teaches detection of analytes using fluorescent energy transfer. The invention provides labeled proteins suitable for determining the presence of cAMP, other second messengers and organic molecules. The proteins are separately labeled with fluorochromes which, when in close spatial proximity, preferably, less than about 6 nm, interact through the transfer of energy from one fluorochrome to the other. Column 4, line 41 to column 8, line 31

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discusses the different formats used and the basis of the radiationless energy transfer. In the method two proteins which are associated in one state and substantially disassociated in another, the equilibrium between being controlled by the free concentration of the analyte. A and D are fluorochromes, the emission wavelength of fluorochrome D overlapping the excitation wavelength of fluorochrome A and the distance between A and D being in sufficiently close proximity to allow the radiationless transfer of energy between the fluorochromes. A and D can be selected from a variety of acceptor, donor pairs such as fluorescein and tetramethylrhodamine and derivatives thereof. Other examples of fluorophore pairs are 7-aminocoumarin and dipyrromethene-borate, an indocarbocyanine and rhodamine X, or a phycoerythrin and allophycocyanin. The concentration of analytes such as cAMP, calcium, cGMP, diacylglycerol, relevant hormone-receptor complexes or an organic molecule such as phorbol ester in a sample can be determined by contacting the sample with (S.sub.1.A).sub.n1 (S.sub.2.D).sub.n2 providing energy near the excitation wavelength of D and measuring the fluorescence of A or D, the concentration of cAMP and other said analytes being determined by the ratio of emission of D to the emission of A, previously calibrated with reference solutions of known analyte concentration. Column 5 lines 29-63 teach the attachment of the respective fluorophores to either of the proteins involved in the analyte controlled complex, the conditions that need to be met for radiationless energy transfer and that the choice of fluorophore pairs are within the skill of one of ordinary skill in the art. In the example given the formation of a holoenzyme is controlled by the presence of the analyte cAMP. Column 6, lines 27-49 teach that in the method a sample is contacted with the labeled proteins; energy near the excitation wavelength of D or A is provided; the fluorescences emitted at the emission wavelengths of A and D respectively is measured; and the extent of energy transfer from D to A is determined by the ratio of their emission amplitudes, or by the fluorescence lifetime of D, or by the rate of photobleaching of D. Any of these parameters, but most conveniently the ratio of emission amplitudes, can be related to the analyte concentration by a calibration curve established with samples of known analyte concentration.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to incorporate a second fluorophore into the Thompson method as taught by Tsien and use a ratio measurement of intensity as taught by Tsien because of its convenience as taught by

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Tsien. Relative to the placement of the donor or acceptor on the carbonic anhydrase, Tsien clearly shows that both possibilities are equivalent and are within the skill of one of ordinary skill in the art as is the selection of the fluorescent energy transfer pairs. Therefore it would have been obvious to one of ordinary skill in the art to use a fluorescent sulfonamide as the donor in the Thompson method because of the equivalence of the equivalence taught by Tsien relative to which fluorophore is the donor and because Thompson clearly shows that fluorescent sulfonamides are known to bond to zinc in the complex.

9. Claims 14-18 and 20 rejected under 35 U.S.C. 103(a) as being unpatentable over Thompson in view of Tsien as applied to claims 14-18 and 20 above, and further in view of Jensen. Thompson does not teach other fluorescent proteins.

In the paper Jensen teaches enhanced fluorescence resonance energy transfer between spectral variants of green fluorescent protein through zinc-site engineering. Although spectral variants of GFP should in theory be suited for fluorescence resonance energy transfer (FRET) and therefore suited for studies of protein-protein interactions, the unfavorable location of the fluorophore 15 Å deep inside the GFP molecule has especially impaired this application. Here, metal-ion site engineering around the dimerization interface of GFP is applied to the cyan and the yellow spectral variant of GFP to stabilize the heterodimeric form of these molecules and thereby increase FRET signaling. The FRET signal, determined as the ratio between the maximal emission for the yellow variant, 530 nm, and the cyan variant, 475 nm, during excitation of the cyan variant at 433 nm was increased up to 8-10-fold in the presence of 10^{-4} M $ZnCl_2$ by engineering of two symmetric metal-ion sites being either bidentate or tridentate. A similar increase in FRET signaling was however obtained in a pair of molecules in which a single bidentate metal-ion site was generated by introducing a zinc-binding residue in each of the two spectral variants of GFP and therefore creating an obligate heterodimeric pair. It is concluded that FRET signaling between spectral variants of GFP can be increased by stabilizing dimer formation and especially by favoring heterodimer formation in this case performed by metal-ion site engineering.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to incorporate other known or modified fluorescent proteins such as those taught by Jensen in the method of Thompson because of their enhance stability and increased emission.

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10. Claims 4, 10 and 19 are rejected under 35 U.S.C. 103(a) as being unpatentable over Thompson '236 in view of Tsien as applied to claims 2 and 16 above, and further in view of Thompson (SPIE 1999, volume 3603, hereinafter referred to as Thompson '99).

In the paper Thompson '99 teaches improved fluorophores for zinc biosensing using carbonic anhydrase. Previous work showed that the zinc-dependent binding of certain fluorescent aryl sulfonamide inhibitors could be used with apo-carbonic anhydrase II to transduce the level of free zinc as a change in the fluorescence of the inhibitor. While inhibitors such as dansylamide (the fluorescent sulfonamide of Thompson '236 figure 1), ABD-M, and ABD-N made possible quantitation of free zinc in the picomolar range with high selectivity, they have only modest absorbance which limits their utility. We describe here the synthesis and properties of two new probes, Dapoxyl sulfonamide and BTCS, and their use in zinc biosensing. Dapoxyl sulfonamide exhibits a dramatic increase and blue shift in its emission upon binding to holo-carbonic anhydrase II, as well as a twenty-fold increase in lifetime: it is thus well suited for quantitating free Zn(II) down to picomolar ranges. The anisotropy of BTCS increases five-fold binding to the holoprotein making this probe well suited for anisotropy-based determination of zinc.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to have used the fluorescent Dapoxyl sulfonamide of Thompson '99 in the Thompson '236 method because of the clear advantages taught by Thompson '99 compared to the prior fluorescent sulfonamides.

11. The prior art made of record and not relied upon is considered pertinent to applicant's disclosure. The additional references relate to carbonic anhydrase and zinc or fluorescent energy transfer methods.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Arlen Soderquist whose telephone number is (571) 272-1265. The examiner can normally be reached on Monday-Thursday and Alternate Fridays.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Jill Warden can be reached on (571) 272-1267. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

A handwritten signature in black ink, reading "Arlen Soderquist". The signature is fluid and cursive, with the first name "Arlen" and last name "Soderquist" clearly distinguishable.

Arlen Soderquist
Primary Examiner
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